Unconventional Circularizable Bacterial Genetic Structures Carrying Antibiotic Resistance Determinants

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Particular genetic structures which—though they lack their own recombinase genes—can be excised in circular form thanks to extensive direct repeats (DRs) flanking the DNA segment undergoing excision have recently been described in both Gram-negative and Gram-positive bacteria (1–6). They carry mostly antibiotic resistance genes. The earliest and the latest three of the above-noted studies were published in *Antimicrobial Agents and Chemotherapy* in 2006 (1) and 2012 (4–6). Although it is probably too early to consider such structures a new group of mobile elements, they are positively unlike conventional mobile genetic elements (MGEs) (plasmids, bacteriophages, integrative and conjugative elements [ICEs], or transposons) (7) and are here tentatively referred to as unconventional circularizable structures (UCSs). Reported UCSs and some putative UCSs are shown in Table 1.

Besides excision, UCS integration in the repaired genetic context has been demonstrated experimentally, suggesting that, once excised, the DNA fragment can not only be lost but also undergo transposition (3). A recent study of eukaryotic genomes (Arabidopsis) hypothesized that intrachromosomal recombination of DRs having nontransposon sequences and subsequent insertion of the circular product may be the predominant mechanism of gene transposition (8). UCSs occurring in bacteria may play a similar role, which their frequent carriage of antibiotic resistance determinants makes even more intriguing. On the other hand, the resulting resistant phenotype could make those UCSs easier to find than UCSs devoid of resistance genes.

The DRs acting in UCSs are usually long—up to more than 100 times longer than the well-established *att* sites acting in conventional MGEs (7)—and imperfect, and they may contain genes (of course, genes not involved in transposition). The encompassed DNA segments vary in length and often carry niche adaptation determinants. The *recA* gene has been shown to be dispensable for UCS excision/integration (2, 3, 6). A parasitic mobilization strategy via site-specific recombination and exploitation of the host *trans*-acting functions has been hypothesized (3), although the possibility of alternative homologous recombination pathways cannot be excluded, nor can the possibility that different UCSs have different mechanisms of excision/integration.

An early report of a genetic structure apparently representing a UCS involved a circular minielement carrying the tetracycline resistance determinant *tet(W)* in the conjugative transposon TnB1230 of *Butyrivibrio fibrisolvens* (1). Remarkably, the minielement was detected in the transconjugants but not in the donor, suggesting that excision was dependent on host functions. Afterwards, two UCSs were characterized in enteric bacteria, one representing a defective prophage (2) and the other a microcin-encoding genomic island (3). Very recent studies of Gram-positive bacteria described UCSs consistently carrying antibiotic resistance genes. One, bearing the multidrug resistance gene *cfr* and containing the macrolide resistance gene *erm*(B) in the DRs, was reported in a methicillin-resistant *Staphylococcus aureus* isolate (4). Two more UCSs were described in streptococci, namely, *Streptococcus suis* and *Streptococcus pneumoniae*. The former (5), carried on an ICE, contains a number of antibiotic resistance genes: *tet(O/W/32/O)* and *tet(40)* (tetracycline), *erm*(B) (erythromycin), *aadE* (streptomycin), and *aphA* (kanamycin). The latter (6) is the well-known MAS (macrolide-aminoglycoside-streptothricin) element, whose insertion distinguishes Tn1545/Tn6003 from Tn6002 (9, 10); again, the DRs contain the *erm*(B) gene.

Of special interest is the involvement of *erm*(B) in recombination events concerning some UCSs. Besides the two *erm*(B)-containing DRs mentioned above (4, 6), *erm*(B)-containing DRs are likely to account for a deleted form (11) of Tn5398, the best-known *erm*(B)-carrying element of *Clostridium difficile* (12). *erm*(B), one of the most prevalent and best-conserved antibiotic resistance genes in bacteria (http://faculty.washington.edu/marilynr/), may enable those UCSs that exploit it for integration to attain diverse, even phylogenetically distant, bacterial genomes.

The antibiotic resistance determinants carried by UCSs are often freshly acquired genes for the host. This is true of *tet(W)* in *B. fibrisolvens* (13), of chromosomally located *cfr* in *S. aureus* (14), of *tet(O/W/32/O)* and *tet(40)* in *S. suis* (5, 15), and of *aphA* and *sat4* (streptomycin) in the MAS element, when the clinical pneumococcus carrying it was originally isolated (16). It also applies to other instances where a UCS is suspected but was not expressly investigated. For example, when *tet(W)* was first described in *Rothia*, it was found in a region flanked by DRs containing a *mef* (macrolide efflux) gene (17). The cat (chloramphenicol acetyltransferase) gene was found in a spontaneously curable cargo DNA region flanked by DRs containing the *toxin/antitoxin* genes when detected in Tn5253 of *S. pneumoniae* (18–20). In addition, *erm(43)*, a new *erm* gene lately identified in *Staphylococcus lentus*, was found in an acquired DNA fragment flanked by DRs (21).

The inherent instability of UCSs makes them unlikely to persist long as such in a given genetic context; rather, they will tend either to become stable (e.g., by sequence divergence between DRs or deletion of either DR) or to be lost (and possibly move to another genetic context). It is reasonable to assume that several resistance determinants have been acquired via UCSs and have later stabilized. The fact that UCSs are often carried by conventional MGEs might entail a mutual benefit, with UCSs contributing to prompt...
renewal of the MGE cargo and with MGEs ensuring interhost spread of UCs

REFERENCES


### Table 1: Characteristics of the reported and of some putative UCs

<table>
<thead>
<tr>
<th>UCS</th>
<th>Bacterial host</th>
<th>Genetic location</th>
<th>Size (kb)</th>
<th>DR length (kb)</th>
<th>% nucleotide identity of DRs</th>
<th>Recognized niche adaptation genes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>tet(W)-containing fragment</td>
<td>Butyrivibrio fibrisolvens</td>
<td>TnB1230</td>
<td>4</td>
<td>0.71</td>
<td>100</td>
<td>tet(W)</td>
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<td>dSE14</td>
<td>Salmonella enterica serovar Enteritidis</td>
<td>Chromosome</td>
<td>12.7</td>
<td>0.31</td>
<td>98</td>
<td></td>
<td>2</td>
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<td>H47 genomic island</td>
<td>Escherichia coli</td>
<td>Chromosome</td>
<td>12.9</td>
<td>0.15</td>
<td>76</td>
<td>Microcin system genes</td>
<td>3</td>
</tr>
<tr>
<td>cfr-containing fragment</td>
<td>Staphylococcus aureus</td>
<td>Plasmid-like insertion</td>
<td>7.5</td>
<td>1.55</td>
<td>100</td>
<td>cfr erm(B)*</td>
<td>4</td>
</tr>
<tr>
<td>15K</td>
<td>Streptococcus suis</td>
<td>ICESu32457</td>
<td>16</td>
<td>1.27</td>
<td>95</td>
<td>tet(O/W32/O) tet(40) erm(B) adde aphA</td>
<td>5</td>
</tr>
<tr>
<td>MAS element</td>
<td>Streptococcus pneumoniae</td>
<td>Tn6003/Tn1545</td>
<td>5.4</td>
<td>1.17</td>
<td>99</td>
<td>apha sat4 erm(B)*</td>
<td>6</td>
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<td>erm(B)-containing fragment b</td>
<td>Clostridium difficile</td>
<td>Tn5398</td>
<td>3.7</td>
<td>1.25</td>
<td>99</td>
<td>erm(B)*</td>
<td>12</td>
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<td>Rothia sp.</td>
<td>Undetermined</td>
<td>12.6</td>
<td>2.13</td>
<td>99</td>
<td>tet(W) mef*</td>
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<td>Streptococcus pneumoniae</td>
<td>Tn5253</td>
<td>9</td>
<td>1.34</td>
<td>95</td>
<td>cat</td>
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<td>erm(43)-containing fragment b</td>
<td>Staphylococcus lentus</td>
<td>Chromosome</td>
<td>6.2</td>
<td>0.13</td>
<td>95</td>
<td>erm(43)</td>
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</tr>
</tbody>
</table>

*a* Contained in the DRs.

*b* Putative UCs.